REMARKS

Reconsideration of the above-identified application in view of the amendment above and the remarks below is respectfully requested.

No claims have been canceled or added in this paper. Claims 1 and 9 have been amended in this paper. Therefore, claims 1-15 are pending. Of these claims, claims 12-15 are withdrawn as being directed at a non-elected invention. Accordingly, claims 1-11 are under active consideration.

In the outstanding Office Action, the Patent Office states the following regarding the Sequence Rules:

> The application fails to comply with CFR 1.821(d), which states:
>
> (d) Where the description or claims of a patent application discuss a sequence that is set forth in the "Sequence Listing" in accordance with paragraph (c) of this section, reference must be made to the sequence by use of the sequence identifier, preceded by "SEQ ID NO:" in the text of the description or claims, even if the sequence is also embedded in the text of the description or claims of the patent application.
>
> For example, page 20 lines 21-22, contains a nucleic acid sequence. Applicant is required to check the rest of the disclosure for any other nucleic acid or protein sequences and list them in a sequence listing and identify them with a proper SEQ ID NO.
>
> The specification and sequence listing must be amended to bring it into sequence compliance. **For any response to this office action to be fully compliant, the response has to bring the application in compliance with sequence rules.** (Emphasis in original.)

In response to the above, Applicant has amended the specification to include the appropriate sequence identifiers. In addition, Applicant is submitting herewith a Sequence Listing that includes

the sequences in question. Accordingly, Applicant respectfully submits that the present application is now in compliance with the Sequence Rules.

Claims 1-6, 9 and 10 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Lopez et al (WO/1999/10540) in view of Pradhan, et al (Journal of Biological Chemistry (1999) volume 274, pages 33002-33010)." In support of the rejection, the Patent Office repeats its reasons of record and then states the following:

> Genomic methylation pattern is interpreted to include tissue specific methylation patterns.
>
> The amendment to the claims to recite hemimethylation requires the presence of a methylated and non-methylated strands.
>
> Lopez et al teaches the amplification of genomic DNA by PCR in the presence of a thermostable DNA methyltransferase (see figure 1 and page 17, lines 26-28) (claim 1) and amplification by single strand displacement amplification and methylation with a DNA methyltransferase (see page 18, line 10-16) for detection. PCR and single strand displacement amplification are interpreted as steps A-C of claim 1. The strands synthesized by chain extension or single strand displacement contain the methylated parent strand and synthesized strand, which is not methylated and thus are hemimethylated. Lopez teaches ^{3}H-s-adenosyl methionine as a methyl donor with a detectable label (see page 4, line 2) (claim 4 and 5). Lopez et al further teaches the use of anchored PCR primers on a solid matrix to create ordered maps (see page 21 lines 2-4) (claim 6). Lopez et al teaches the treatment of amplified targets with a restriction enzyme capable of distinguishing methylated and non-methylated cytosines (see page 32, lines 25-29).
>
> Lopez et al does not teach the use of DNA methyltransferase that preserves methylation status of genomic DNA (claim 1). Lopez et al does not teach the use of DNMT1 a maintenance methyltransferase (claims 2 and 3).
>
> However, Pradhan et al teaches the use of DNMT1 as a methyltransferase (see abstract). Pradhan teaches maintenance methylation "ensures propagation of tissue specific methylation patterns during development" (see page 33002, first column text,

lines 8-10). Pradhan teaches that DNMT1 has a higher reaction velocity for hemimethylated DNA substrates (see page 3302, 2nd column, last paragraph). Pradhan thus teaches DNMT1 is a maintenance methyltransferase ensures propagation of specific methylation patterns. Pradhan further teaches cytosine methylation is important in embryonic development, carcinogenesis and genetic disease (see page 33002, 1st column of text lines 1-5). Pradhan thus teaches maintenance methylation and the methyltransferases that maintain methylation patterns are important in embryonic development, carcinogenesis and genetic disease.

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the DNMT1 methyltransferase taught by Pradhan as the methyltransferase in Lopez's method because Pradhan teaches DNMT1 is a maintenance methyltransferase that ensures propagation of methylation patterns. The ordinary artisan would be motivated to use the DNMT1 of Pradhan with Lopez method of methylating amplified DNA because Pradhan maintenance methylation and the methyltransferases that maintain methylation patterns are important in embryonic development, carcinogenesis and genetic disease. The artisan would have a reasonable expectation of success as they are merely replacing a one methyltransferase for another.

The Patent Office then continues as follows:

The response of 3/5/2008 asserts in the first line of page 7 that the present invention teaches, "a method for use in METHYLATION analysis." This is noted, however the invention as claimed is drawn to a method of amplifying genomic DNA is a manner in which methylation status is maintained.

The response further asserts on the top of page 8, the PCR amplification of Lopez "erases all existing methylation information." This argument has been thoroughly reviewed but is not considered persuasive as PCR amplification does not result in a loss of methylation information, as the methylation on the genomic DNA is still intact, not erased. Thus the assertion that the method of Lopez "erases all existing methylation information" is incorrect and thus not persuasive.

The response further asserts the method of detecting would be unreliable if partially methylated DNA is produced. This argument has been thoroughly reviewed but is not considered persuasive as

> Lopez does not teach that the genomic methylation status is lost. Lopez is being used to merely teach that methods of amplifying genomic DNA by the use of PCR which requires heating, cooling and primer extension were known.
>
> The response further asserts, "Lopez teaches a completely different problem in a completely different technology." This argument has been thoroughly reviewed but is not considered persuasive as Lopez is drawn to a method of amplifying genomic DNA and analyzing methylation status by use of a methyl transferase. The instant invention is drawn to the use of amplifying genomic DNA using a methyltransferase. Thus the methods while directed to slightly different outcomes both use amplification of genomic DNA and a methyltransferase. The difference between the Lopez and the instant method is that the claimed method requires the use of a methylation sensitive methyltransferase, such as DNMT1 taught by Pradhan, which will maintain methylation status of the genomic DNA. Thus the difference is the substitution of a single reagent. It is noted that simply replacing the methyltransferase of Lopez with the DNMT1 and using DNMT1 would result in an amplification of a plurality of different of nucleic acids with their methylation patterns intact as each initially amplified strand would result in the production of a complement in which methylation was maintained.
>
> However, it would have also been obvious to the ordinary artisan to add a DNMT1 after each heating, cooling and primer extension cycle as Pradhan teaches the protein is labile and thus subject to degradation at moderate temperatures (see page 33005, 1st column, 1st full paragraph).

Applicant respectfully traverses the subject rejection.

Claim 1, from which claims 2-6, 9 and 10 depend, has been amended in this paper and now recites "[a] method for the amplification of genomic DNA whereby the cytosine methylation pattern of the genomic DNA is retained in the amplificate sequence(s), said method comprising the following steps:

(a) providing a sample of DNA, said DNA being methylated at one or more cytosine positions;

(b) heating the genomic DNA to a temperature operative to cause denaturation;

(c) cooling the denatured DNA in the presence of single stranded oligonucleotide primers such that the primers anneal to the DNA;

(d) heating the mixture in the presence of a polymerase and nucleotides to a temperature such that the primers are extended, thereby resulting in hemimethylated DNA;

(e) contacting the hemimethylated DNA with a methyltransferase and a methyl donor molecule under conditions conducive to the methylation of the synthesised strand such that the CpG dinucleotides within the synthesised strand are methylated according to the methylation status of the corresponding CpG dinucleotide on the template strand thereby preserving the genomic methylation pattern;

(f) repeating steps (b)-(d) a plurality of times to reach a plurality of nucleic acids, whereby each of said nucleic acids is methylated at the same one or more cytosine positions as the DNA provided in step (a); and

(g) analyzing the methylation of the nucleic acids of step (f) whereby the methylation of the DNA of the sample of step (a) is deduced."

Support for the present amendment to claim 1 may be found in the present specification, for example, on page 19, lines 13-17 (with respect to new step (a) of claim 1); on page 10, lines 20-25, and page 11, lines 1-18 (with respect to amended step (f) of claim 1); and on page 6, lines 8-10 (with respect to amended step (g) of claim 1).

Claim 1 is patentable over Lopez in view of Pradhan because it would not have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the DNMT1 methyltransferase of Pradhan as the methyltransferase in the method of Lopez. This is at least

because (a) a person of ordinary skill in the art would have had no reasonable expectation of success in combining the teaching of Lopez and the teaching of Pradhan; (b) the presently claimed method and the method of Lopez are not of analogous fields; and (c) the combination of Lopez and Pradhan does not teach all of the limitations of the presently claimed method.

With respect to the first point above, a person of ordinary skill in the art would have had no reasonable expectation of success in combining the teaching of Lopez and the teaching of Pradhan. It is a requirement of Lopez's method that the applied methyltransferase is specific for a recognition site (e.g., claim 1, p. 14, line 34 – p. 15, line 6). Lopez's method is based thereon that a single nucleotide point mutation or polymorphism creates or destroys the sequence-specific recognition site of the DNA methyltransferase. The presence or absence of methylation is then detected on which the sequence variance is then deduced (p. 14, line 34 – p. 15, line 6). However, this is not possible according to the teachings of Pradhan. According to Pradhan, DMNT1 methyltransferase is specific for several recognition sites, i.e., CG, FG, FCG, or FWG (wherein F is 5-fluoro-2'-deoxycytidine and W is either T or A). Thus, for example, in case of a C to T mutation, DMNT1 would not allow a differentiation between the recognition sites FCG, FTG or FAG. In addition, an insertion of C, T or A into the recognition sequence FG could not be detected by the DMNT1 methyltransferase applied to Lopez's method. Because of this, a person of ordinary skill in the art would have had no reasonable expectation of success in combining the methods of Lopez and Pradhan.

In addition, the ordinary artisan would have had no reasonable expectation of success in combining the teachings of Lopez and Pradhan because, according to Lopez, DNA may be amplified and methylated in subsequent reactions or within the same reaction mixture (p. 17, lines 15-33, Fig. 1, p. 18, lines 10-16, Fig. 2). In case of subsequent reactions, unmethylated DNA is first amplified

and the amplified DNA is then methylated (p.17, lines 15-23). Because cytosine and methyl-cytosine have the same base pairing behaviour, PCR amplified DNA is unmethylated. Thus, the subsequent methylation is a de novo methylation of both strands. In the other case, amplification and methylation take place in the same reaction mixture, is illustrated by Fig. 1 and Fig. 2. According to Fig. 1 and 2, DNA unmethylated at a recognition site of interest is first amplified, whereby double stranded unmethylated DNA is generated. The double stranded DNA is then methylated by a methyltransferase. Because the amplified DNA is unmethylated, methylation is a de novo methylation. Pradhan, on the other hand, teaches that DMNT1 has a 7-21 fold preference of hemimethylated over unmethylated DNA (Abstract). In addition, Pradhan cites Kho et al., according to which the preference is 134 fold (p. 33009, left col., last paragraph). Because Lopez teaches a de novo methylation and Pradhan teaches, although in principle possible, DMNT1 is inappropriate for de novo methylation, the ordinary artisan would have had no reasonable expectation of success in combining the teachings of Lopez and Pradhan.

Consequently, because the ordinary artisan would have had no expectation of success in combining the method Lopez and Pradhan, the instant method is not prima facie obvious over Lopez in view of Pradhan.

With respect to the second point above, the inventive method is not prima facie obvious over Lopez in view of Pradhan because Lopez's method and the presently claimed method are not of analogous fields. The presently claimed method is a method for the amplification of nucleic acid (preamble, claim 1, p. 5, lines 25-28). In contrast thereto, the method of Lopez is a method for identifying a DNA genotype (p. 1, line 10, preamble of claim 1). As a result, "genotype" refers to a particular combination of sequence-specific sites present or absent in an individual genome or

fragment of DNA (p. 15, lines 10-11). Thus, Lopez teaches a method for determining the succession of nucleic acid bases (i.e., A, C, T, G) while it is subject matter of the instant application to amplify methylated DNA. Because the two fields are unrelated to each other, the two methods are not of an analogous field.

With respect to third point above, the presently claimed method is not prima facie obvious over Lopez in view of Pradhan because the combination of Lopez and Pradhan does not teach all of the limitations of the presently claimed method. Neither Lopez nor Pradhan teaches the limitation of claim 1 of providing a sample of DNA, wherein the DNA is methylated at one or more cytosine positions. Instead, according to Lopez, the DNA provided is methylated by a methyltransferase. Subsequently, the transferred methyl-groups are detected wherefrom the sequence of nucleic acid bases is deduced. This implies that the recognition site of the methyltransferase is unmethylated. Otherwise, methylation is not possible and, thus, the identifying of the DNA genotype. This is also confirmed by the following passages and Figures of Lopez: p. 4, lines 1-13; p. 17, lines 15-23; Fig. 1; and Fig. 2.

Also, Pradhan does not teach providing methylated DNA. Instead, according to Pradhan, unmethylated and hemimethylated DNA are provided. Because the combination of Lopez and Pradhan does not teach providing DNA methylated at one or more cytosine positions, the instant method is not prima facie obvious over Lopez in view of Pradhan.

In addition, neither Lopez nor Pradhan teaches the claimed limitation of analyzing the methylation of amplified DNA whereby the methylation of the provided DNA is deduced. In fact, Lopez does not address methylation analysis of the provided DNA at all. Instead, according to Lopez, provided DNA is methylated; subsequently, this introduced methylation is analyzed

wherefrom a deduction is made on the base sequence (i.e. the sequence of the bases A, C, T, and G) of the provided DNA. However, Lopez is silent about the determination of the methylation of the provided DNA. Similarly, Pradhan is silent about the analysis of methylation of provided DNA. According to Pradhan, DNA is only provided to characterize the enzymatic properties of DNMT1 (e.g., description of Fig. 3, Fig. 4, Fig. 5, Table 1). Because the combination of Lopez and Pradhan does not teach analyzing the methylation of the provided DNA, the instant method is not prima facie obvious over Lopez in view of Pradhan.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 7 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over Lopez et al (WO/1999/10540) in view of Pradhan, et al (Journal of Biological Chemistry (1999) volume 274, pages 33002-33010) as applied to claim 1-6, 9, and 10 above, and further in view of Shatkin et al (US Patent 6312926)." In support of the rejection, the Patent Office states the following:

> The teachings of Lopez and Pradhan are set forth above. Lopez and Pradhan do not teach the methyltransferase immobilized on a solid support.
>
> However, Shatkin et al teaches the use of hMET (methyl transferase) immobilized on protein G beads for washing assays (see column 24, lines 3-12).
>
> Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve Lopez and Pradhan's method of amplifying genomic DNA while maintaining genomic methylation patterns with immobilized methyltransferase taught by Shatkin, because Shatkin teaches immobilization allows washing of assays. The ordinary artisan would be motivated to improve Lopez and Pradhan's method of amplifying genomic DNA while maintaining genomic methylation patterns with immobilized methyltransferase or polymerases as taught by Shatkin, because Shatkin teaches immobilization allows washing of assay and detection of protein interactions.

The Patent Office then continues as follows:

> The response of 3/5/2008 asserts that Shatkin et al does not cure all of the deficiencies of Lopez in view of Pradhan, as previously presented in the response. These arguments have been thoroughly reviewed but are not considered persuasive because as discussed above Lopez in view of Pradhan does render the instant claims obvious as the combination would result in a method of amplifying genomic DNA wherein the methylation status of the genomic DNA is maintained. The response does not set forth any other arguments to this rejection, thus this rejection is maintained.

Applicant respectfully traverses the subject rejection. Claim 7 depends from claim 1. Claim 1 is patentable over the combination of Lopez et al. and Pradhan et al. for at least the reasons given above. Shatkin et al. fails to cure all of the deficiencies of Lopez et al. and Pradhan et al. with respect to claim 1. Therefore, based at least on its dependency from claim 1, claim 7 is patentable over the applied combination of Lopez et al., Pradhan et al. and Shatkin et al.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 8 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over Lopez et al (WO/1999/10540) in view of Pradhan, et al (Journal of Biological Chemistry (1999) volume 274, pages 33002-33010) as applied to claims 1-6, 9, and 10 above, and further in view of Stemple et al (WO/2000/53805)." In support of the rejection, the Patent Office states the following:

> The teachings of Lopez and Pradhan are set forth above. Lopez and Pradhan do not teach the polymerase immobilized on a solid support.
>
> However, Stemple teaches the immobilization of a polymerase on a solid support (see page 3 lines 14-15). Stemple teaches immobilization or fixing the site of the polymerase allows assaying of multiple nucleic acids simultaneously (See page 7, lines 25-26).
>
> Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve Lopez and Pradhan's method of amplifying genomic DNA while

> maintaining genomic methylation patterns with immobilizing a polymerase as taught by Stemple, because Stemple teaches immobilization or fixing the site of the polymerase allows assaying of multiple nucleic acids simultaneously. The ordinary artisan would be motivated to improve Lopez and Pradhan's method of amplifying genomic DNA while maintaining genomic methylation patterns with immobilized polymerases as taught by Stemple, because Stemple teaches immobilization or fixing the site of the polymerase allows assaying of multiple nucleic acids simultaneously.

The Patent Office then continues as follows:

> The response of 3/5/2008 [asserts] that Stemple et al does not cure all of the deficiencies of Lopez in view of Pradhan, as previously presented in the response. These arguments have been thoroughly reviewed but are not considered persuasive because as discussed above Lopez in view of Pradhan does render the instant claims obvious as the combination would result in a method of amplifying genomic DNA wherein the methylation status of the genomic DNA is maintained. The response does not set forth any other arguments to this rejection, thus the rejection is maintained.

Applicant respectfully traverses the subject rejection. Claim 8 depends from claim 1. Claim 1 is patentable over the combination of Lopez et al. and Pradhan et al. for at least the reasons given above. Stemple et al. fails to cure all of the deficiencies of Lopez et al. and Pradhan et al. with respect to claim 1. Therefore, based at least on its dependency from claim 1, claim 8 is patentable over the applied combination of Lopez et al., Pradhan et al. and Stemple et al.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 11 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over Lopez et al (WO/1999/10540) in view of Pradhan, et al (Journal of Biological Chemistry (1999) volume 274, pages 33002-33010) as applied to claims 1-6, 9 and 10 above, and further in view of Gonzalgo et al (US Patent 6251594)." In support of the rejection, the Patent Office states the following:

> The teachings of Lopez and Pradhan are set forth above. Lopez and Pradhan do not teach the use of bisulphate solution to distinguish methylation status of cytosine bases.
>
> However, Gonzalgo et al teach the use of bisulphite to distinguish methylated and unmethylated cytosines (column 7, lines 5-6). Gonzalgo teaches the use of bisulphite is quantitative, does not use restriction enzymes, and allows multiplexing (see column 7, lines 7-10).
>
> Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve Lopez and Pradhan's method of amplifying genomic DNA while maintaining and distinguishing genomic methylation patterns by use bisulphite solutions taught by Gonzalgo, because Gonzalgo teaches the use of bisulphate is quantitative, does not use restriction enzymes, and allows multiplexing. The ordinary artisan would be motivated to improve Lopez and Pradhan's method because, the use of bisulphite is quantitative, does not use restriction enzymes, and allows multiplexing. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the instant invention was made, it must be considered that said ordinary skilled artisan would have had reasonable expectation of success in practicing the claimed invention.

The Patent Office then continues as follows:

> The response of 3/5/2008 [asserts] that Gonzalgo et al does not cure all of the deficiencies of Lopez in view of Pradhan, as previously presented in the response. These arguments have been thoroughly reviewed but are not considered persuasive because as discussed above Lopez in view of Pradhan does render the instant claims obvious as the combination would result in a method of amplifying by PCR genomic DNA wherein the methylation status of the genomic DNA is maintained. The response does not set forth any other arguments to this rejection, thus this rejection is maintained.

Applicant respectfully traverses the subject rejection. Claim 11 depends from claim 1. Claim 1 is patentable over the combination of Lopez et al. and Pradhan et al. for at least the reasons given above. Gonzalgo et al. fails to cure all of the deficiencies of Lopez et al. and Pradhan et al.

with respect to claim 1. Therefore, based at least on its dependency from claim 1, claim 11 is patentable over the applied combination of Lopez et al., Pradhan et al. and Gonzalgo et al.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

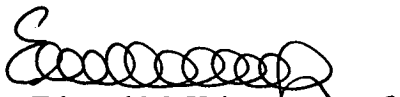
In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is

required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

Kriegsman & Kriegsman

By: 

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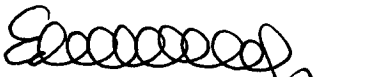

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